Radiation and Chemical Injury in the Bone Marrow

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Hematopoietic system toxicity is a major limiting factor in the use of aggressive combined modality therapy in the treatment of malignant disease. In this review, the known drug-x-ray interactions using tissue culture systems are extended to the bone marrow compartment. Two hypotheses prevail for late bone marrow failure: (1) stromal damage to the vasculature with subsequent fibrosis and (2) irreversible stem cell depletion in the irradiated site.

Clinical extensions of the experimental data for bone marrow kinetics in the animal model have not proven successful to date. The future strategies for therapy of malignancies in which both radiation and chemotherapy are employed may require dose modification or treatment planning to limit bone marrow toxicity.

Introduction

Current approaches to the successful management of malignancies include combined modality treatment. The ability of radiation therapy to control localized disease and of chemotherapy to control micrometastases suggests a complimentary nature for the two modalities. Using this principle and the possibility of potentiation of therapeutic effects, combined modality efforts have been tested in a variety of clinical situations. Diseases in which this approach has been most successful include pediatric, hematological, breast, and gastrointestinal malignancies (1).

Enthusiasm over early results; however, must be tempered by an increased acute toxicity and the potential late effects of combined therapy. Hematopoietic system toxicity is a major limiting factor when aggressive combined therapy is employed. Clinically significant bone marrow injury may result in acute changes such as depletion or depopulation of stem cells and resultant organ failure, or late effects contributing to malignant transformation. Drug injury to the bone marrow has been additive

to radiation injury. By combining radiation therapy and chemotherapy, increased toxicity rather than an improvement in the therapeutic ratio may be observed (2).

In this review we intend to consider the known radiation-tissue interactions, the influences of drugs on these interactions, characterization of bone marrow injury, and laboratory-clinical correlations. Animal data for bone marrow investigations providing insight into these interactions will be reviewed and compared to interactions in other mammalian tissue culture systems. The feasibility of using this current knowledge in developing therapeutic strategies for drug x-ray sequencing will be discussed.

Physics and Radiobiological Considerations

The basic radiation-matter interaction for ionizing radiation in the therapeutic energy range consists of Compton scattering of an x-ray photon and an orbital electron (Fig. 1). Subsequent interactions result in deposition of energy by the scattered electron in critical "target sites" in the cell. These are generally believed to be interactions with DNA. At the cellular level there remain many gaps in the knowledge of mechanism of radiation injury. Theoretical models based on microdosimetric considerations and on DNA breakage results in a

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tion response of cells (3, 4). From a practical viewpoint, however, most data is analyzed using classical target theory (5).
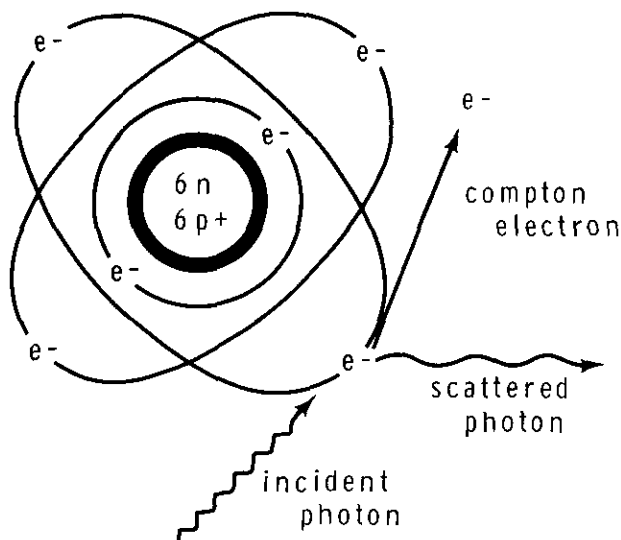

FIGURE 1. Radiation-matter interaction illustrating an incident photon (x-ray) interacting with a loosely bound electron giving rise to the Compton electron.

The cell cloning and colony forming techniques originally described by Puck and Marcus in 1956 have led to semilogarithmic dose response curve for mammalian cells and to a concept of log kill with radiation (6). The concept is based on cells losing or maintaining the capacity for unlimited division to form colonies. Using a different technique and chemotherapeutic agents, Skipper et al. developed the concept of fractional kill in 1964 (7). In practice, the two approaches represent a single concept with implications for cure of disease and tolerance to treatment.

Since the mechanism of radiation injury and repair are not well established, much of the characterizations of radiation-cell interactions are based on functional definitions of radiation damage and repair using the colony forming assay systems. In particular, mammalian cells have been shown to demonstrate two operationally different repair processes.

Sublethal damage (SLD) repair is expressed as the increase in survival observed as the interval between two doses is increased (8). Potentially lethal damage (PLD) repair is expressed by the increase in survival following a single dose when post-irradiation conditions are appropriate (9). Figure 2 illustrates a proposed hierarchy of radiation

No Damage ⇌ SLD ⇌ PLD → Lethal Damage

FIGURE 2. Interrelationship of levels of radiation injury. SLD-sublethal radiation damage, PLD-potentially lethal radiation damage.

damage. A cell may skip an intermediate damage level when injury or repair processes occur (10).

The actions of chemotherapeutic agents on cells may result in exponential or biphasic killing curves (11). Actions of drugs and radiation on cells may result in these categories: (1) additivity, i.e., independent actions of drug and radiation are combined; (2) potentiation, i.e., nontoxic doses of a drug combined with radiation results in enhancement of radiation damage; and (3) synergism, i.e., the combined effect is greater than the simple addition of actions of the individual modalities. The radiation survival curve may be changed (decrease the capacity of cells to accumulate SLD) or the ability of cells to repair SLD or PLD may be impaired (12, 13). Table 1 summarizes known drug-radiation interactions for several frequently used chemotherapeutic agents. A "recall" phenomenon is observed when there is an enhanced reaction in an irradiated volume on treatment with drugs weeks following completion of irradiation.

Although radiation cell killing occurs in all phases of the mitotic cycle, cells exhibit varying degrees of sensitivity throughout the cycle. Figure 3 illustrates that cells are most sensitive in G_2M phase and least sensitive in late S phase (14). Cell cycle-specific drugs may be more effective (or more toxic) if appropriate drug-x-ray sequencing is used.

Table 1. Drug–radiation interactions.

Drug	X-ray interaction		
	Potentiate	Repair inhibition	"Recall"
Alkylating agents			
Cyclophosphamide	+		
Melphalan			
Antimetabolite			
5FU	+		
Methotrexate	+		+
Antitumor antibiotics			
Adriamycin	+	+	+
Bleomycin	+	+	+
Actinomycin D	+	+	+
Miscellaneous			
Vincristine	+		
BCNU	+		
Cis-Platinum	+	+	
Hydroxyurea	+		

Environmental Health Perspectives

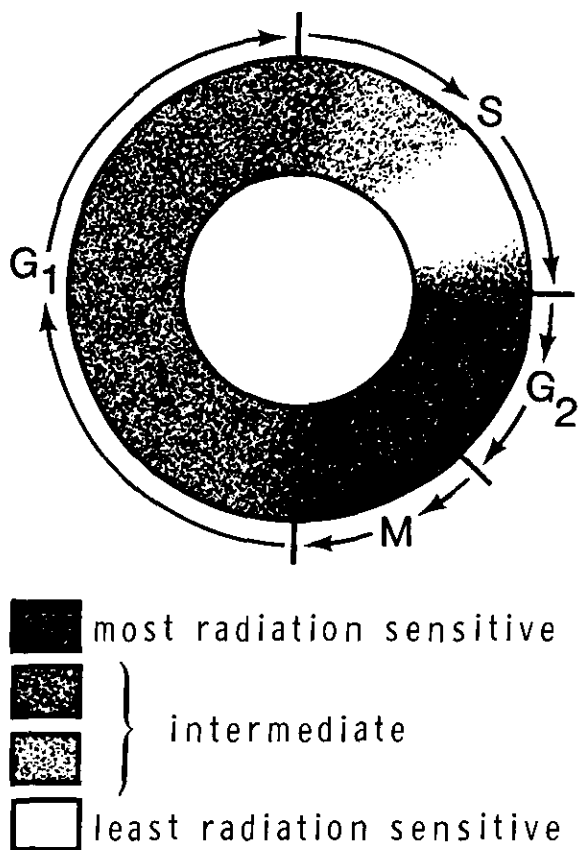FIGURE 3. Relative radiation sensitivities of mammalian cells in the stages of the mitotic cycle (14).

most radiation sensitive

} intermediate

least radiation sensitive

FIGURE 4. Radiation dose response curve for mouse bone marrow cells using the spleen colony assay system. From Till and McCullough (15, 16).

Radiation Effects on Bone Marrow

Much has been written on the effects of irradiation on hematopoietic tissues. The assay on which much of the experimental data is based is the spleen colony method of Till and McCulloch (15, 16). Usually donor mice undergo variable radiation or drug treament and recipients undergo lethal irradiation. Bone marrow is transplanted and maroscopic spleen colonies are counted 8-10 days later. Figure 4 illustrates data of Till and McCulloch showing the radiation survival curve for mouse bone marrow stem cells (CFU-s).

The ability of the bone marrow compartment to recover and regenerate following irradiation is principally dependent on two factors, the dose of radiation and the volume of bone marrow within the irradiated field (17-19). The acute depletion of bone marrow components following irradiation have been ascribed to the direct effect of radiation depleting the stem cell compartment, whereas the chronic bone marro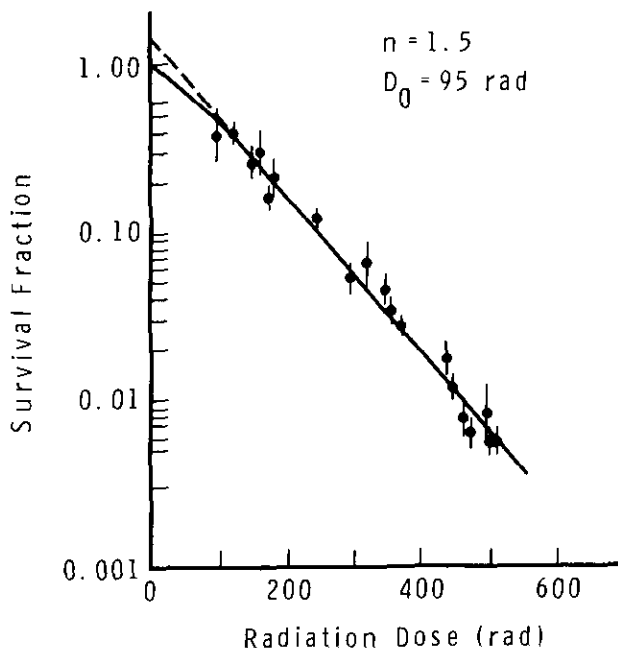w aplasia following initial repopulation has been ascribed to stromal damage thought secondary to an impaired vascular supply and subsequent fibrosis. This has been attributed to endothelial cell damage. The delay in the expression of damage being the result of the prolonged cell division in the stroma.

An alternate hypothesis of late marrow failure has been an irreversible stem cell depletion within the irradiated site. The prime argument against such an hypothesis has been the observation that migrating stem cells are capable of repopulating irradiated bone marrow as demonstrated with whole body irradiation when portions of bone marrow are shielded (20, 21). Questions have been raised to the efficacy of this migrating stem cell pool, both in the total numbers of circulating CFU's available, and in their ability to maintain the capacity for unlimited self renewal as would be required of a true stem cell. Hellman et al. have shown in C3H/HEJ mice that the circulating stem cell pool is very small, perhaps as few as 100, and is rapidly exhausted following radiation (22). Micklem et al. have shown that circulating stem cells show low proliferative potential and cease to multiply after a certain number of divisions (23). Additionally, little migration of stem cells normally occurs between the marrow of different bones (24, 25). Functional studies of the ability of the bone marrow to support clonigenic proliferation have shown a dose related

decrease at all times following radiation (26). Acute recovery following radiation and subsequent late marrow failure does not disprove the stem cell depletion concept.

Despite initial acute recovery in the bone marrow following single doses of radiation as high as 6000 rad, the bone marrow is seen to become aplastic at late times post radiation. The degree and time interval to aplasia is dependent on the dose of radiation (27).

Conventionally, fractionated doses of radiation of greater than 3000 to 4000 rad as observed by Sykes

FIGURE 5. Distribution of bone marrow in the adult (35, 36).

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have been shown to cause permanent aplastic changes in the bone marrow in over 90% of such treated patients (28, 29).

Attempts have been made to artificially repopulate irradiated bone marrow sites with presumably viable stem cells by means of intravenous bone marrow infusion (30). Animal studies have generally been unsuccessful in showing any lasting benefit to irradiate sites using this technique. Knospe gave bone marrow infusion 10 days or 3 months following irradiation without evidence of benefit, although quantative measurements of cellularity or CFU were not attempted in these experiments.

Recent data by Buachidze using autologous bone marrow transplantation in patients receiving total nodal irradiation for Hodgkin's disease have been presented (31). An initial acute benefit was shown for patients who received bone marrow transplantation in both platelet and granulocyte counts during and shortly following irradiation. No information has yet been persented as to the longevity of these acute early responses to marrow transplantation. Acute depletion of the bone marrow failure at sufficient doses would appear to be due both to stromal damage secondary to impairment of the vascular supply as well as to the direct effect of radiation on depletion of the stem cell compartment. This localized stem cell depletion and stromal damage cannot at this time be replenished either with endogenous circulating stem cells or the exogenous infusion of donor stem cells within the irradiated sites.

Clinical Evaluation of Bone Marrow Toxicity

Although drugs effect the entire bone marrow volume, radiation effects are primarily confined to treated volumes. The fraction of bone marrow that will undergo irradiation may be estimated for a planned treatment course. Figure 5 presents the

Table 2. Proportion of bone marrow irradiated by usual therapeutic techniques.

Radiation technique	Estimated bone marrow affected, %
Total body irradiation	100
Total nodal irradiation	60-70
Mantle	20-50
Para-aortic	20-25
Pelvic	15-25
Pulmonary and mediastinal	20-25
Abdominal	20-25
Cranial	25-45
Cranio-spinal	60-75
Chest wall and lymphatic	15-20
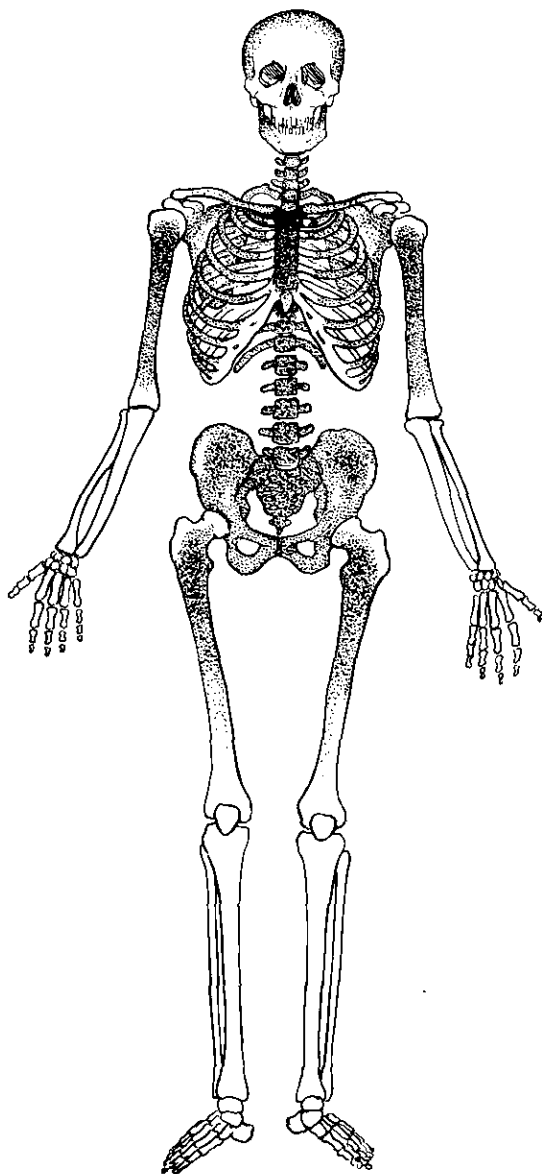
Reference	No. of patients	Evaluation method	Dose (rad)	Observations
Sykes et al. (*28*)	61	Sternal marrow histology	1000-2400	Complete recovery 5/5
			3000-6000	Partial recovery 2/56
Slanina et al. (*33*)	73	Sternal marrow histology	3000-5000	Normal 4%
				Hypoplastic 16%
				Aplasia 80%
	89	Iliac crest marrow histology	Unirradiated	Normal 53%
				Hypoplasia 47%
				Aplasia 0
Rubin et al. (*19*)	27	^{99m}Tc-S Scan	4000	Recovery at 1 year 50%
				Recovery at 2-3 years 80%
Knospe et al. (*34*)	26	^{52}Fe Scan	4000-4400	Marrow volume expression
				0-12 months 8/10
				12-24 months 7/8
				>24 months 1/8

data of Hashimoto and Atkinson illustrating that in the adult, truncal bones and proximal extremities contain the majority of the active bone marrow (*35, 36*). The fractional volumes of bone marrow included in the usual portal for various sites of irradiation are presented in Table 2. As may be observed, significant marrow fractions are in the radiation fields for most diseases in which radiation and chemotherapy play a role and may relate to subsequent toxicity and poor tolerance when drugs are required. To some degree, more careful treatment planning, to minimize bone marrow irradiated, may improve this situation.

The use of animal data may certainly provide insight into basic principles; however, most animal studies are performed with large single dose exposures and are of limited value in predicting human bone marrow regeneration. Table 3 summarizes *four reports of clinical evaluations of bone marrow* following radiation therapy.

Although these studies were performed by evaluating bone marrow histology, or radioisotope uptake, they do provide a basis for several clinically important conclusions: (1) there is a high incidence of aplasia at doses above 3000 rad; (2) results in the peripheral blood may not directly correspond to bone marrow observations (*33*); and (3) the kinetics of regeneration of bone marrow in humans are not established, and may be important for combined modality treatment considerations.

Future Prospects

Experimental results in the animal system do not directly correlate to clinical observations; however, mechanisms and generalizations may be formulated. The goal will be to apply these concepts to attain optimal timing, sequencing, and dosages of radiation and drugs. Attempts to use infusion of autologous bone marrow have not provided any major benefits at this time. As our knowledge and technical capabilities for bone marrow transplantation increase, this may become a reasonable option. In the immediate future, optimal sequencing of drugs and irradiation, along with dose modification appears to be the most feasible approach to limiting bone marrow toxicity.

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